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Attorney's Docket No.: 10276-029001 / JDP-044

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant : Bonner-Weir et al.
Serial No. : 09/602,508
Filed : June 23, 2000
Title : METHODS OF MAKING PANCREATIC ISLET CELLS

Art Unit : 1651
Examiner : V. Afremova

MAIL STOP AF
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION UNDER 37 C.F.R. §1.132 OF DR. SUSAN BONNER-WEIR

I, Susan Bonner-Weir, a citizen of USA, residing in Cambridge, MA, hereby declare as follows:

1. I am a Senior Investigator in the Section on Islet Transplantation and Cell Biology at Joslin and Associate Professor of Medicine at Harvard Medical School. I received my doctorate in biology at Case Western Reserve University and then completed postdoctoral training in islet morphology at Harvard Medical School. I serve or have served on the editorial boards of the American Journal of Physiology, Endocrinology and Diabetes, and was a member of the Juvenile Diabetes Research Foundation Scientific Review Committee.

2. I am a co-inventor of the invention claimed in the above-identified patent application, and I have read and understand the contents of the present patent application.

3. I have been advised and understand that the Examiner has rejected claims of the above-referenced application that are directed to a method of obtaining pancreatic islet cells. The

CERTIFICATE OF MAILING BY FIRST CLASS MAIL

I hereby certify under 37 CFR §1.8(a) that this correspondence is being deposited with the United States Postal Service as first class mail with sufficient postage on the date indicated below and is addressed to the Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

January 8, 2004
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Maria Keen
Signature

Maria Keen
Typed or Printed Name of Person Signing Certificate

method includes: (a) providing differentiated or adult pancreatic cells substantially free of islet cells, (b) allowing the differentiated or adult pancreatic cells to proliferate to form a population of dedifferentiated pancreatic cells, (c) adding a component of extracellular matrix (ECM) to the population of dedifferentiated pancreatic cells; and (d) growing the cells in the presence of ECM for a time sufficient for the cells to express insulin. I further have been advised and understand that this rejection is based, in part, on the Examiner's assertion that the claims are obvious in view of Gmyr et al. [IDS-AG]. The Examiner interprets the Gmyr et al. method "C" as disclosing a step of obtaining dedifferentiated cells and a step of culturing dedifferentiated cells in the presence of ECM. Thus, the Examiner argues, the Gmyr method "C" results in pancreatic islet cells just as in the claimed methods.

4. The Examiner's interpretation of Gmyr is incorrect. Gmyr (as indicated by Gmyr's title) relates to expansion of ductal cells from various types of tissue. Gmyr does not suggest or even speculate that insulin-producing islet cells (as required by the claims) are produced by method "C". Indeed, Gmyr acknowledges that the conditions necessary to produce insulin-producing (endocrine) cells from exocrine or ductal cells are unknown when Gmyr states: "We are now screening various conditions to induce the endocrine differentiation of these ductal precursor cells" (emphasis added). Gmyr does not even speculate about what such conditions might be. Since ductal cells do not produce insulin, the Gmyr cells cannot reasonably be expected to be the same as Applicants' dedifferentiated cells, which Applicants have shown to form islet buds and express insulin upon contacting with a component of ECM.

5. The Examiner's argument, at least in part, appears to be based on the fact that both Gmyr and the claims use the term "dedifferentiated cells". However, this term as used by Gmyr means something completely different than the term as used in the present specification and claims. "Dedifferentiated cells" as defined in the specification and recited in the claims, are adult or differentiated pancreatic cells, substantially free of islet cells, that have been allowed to proliferate, have lost their differentiated phenotype, and are pluripotent. (See specification at

page 2, lines 1-5; page 17, lines 13-15; page 32, line 6 et seq.) As now amended, the claims recite a step of allowing the adult or differentiated cells to proliferate. In contrast, as used by Gmyr, "dedifferentiated cells" are ductal cells that have arisen from a population of exocrine cells. Gmyr describes method C as follows:

> exocrine cells [i.e., acinar cells] were allowed to dedifferentiate to a ductal phenotype in culture. (. . .) the cell yield appeared virtually unlimited (. . .) Immunohistochemical studies confirmed the ductal epithelial (cytokeratin 19, CA 19-9) nature of the cells. (. . .) [Method C] allowed further expansion of ductal epithelial cells when cultured in 804G matrix with HGF or EGF. Conclusion: (. . .) exocrine tissue dedifferentiation in culture allowed the obtention of sufficient numbers of human ductal pancreatic cells and their further in-vitro expansion. We are now screening various conditions to induce the endocrine differentiation of these ductal precursor cells. (Emphasis added.)

Thus, in what Gmyr calls "method C", Gmyr describes the expansion of ductal cells from exocrine tissue. Gmyr refers to the expansion of ductal cells from exocrine tissue in culture as "dedifferentiation." However, it is clear in the field of pancreatic cell culture that **"dedifferentiation" as used by Gmyr, does not include proliferation**. In fact, the *in vitro* expansion of ductal cells from exocrine (acinar) tissue has been shown by others to not involve cell division. This is evidenced by Rooman et al. (2000) *Diabetes* 43:907-914 (enclosed herewith). Although Rooman was published after the present application was filed, it discusses references published prior to the filing date, which describe the obtention of ductal cells from cultured exocrine pancreas. (See Rooman, page 912, first sentence of Discussion) Rooman concludes as follows:

> a nine fold increase in cells with ductal characteristics [from acinar exocrine tissue] with 26 to 64% preservation of initial DNA, and **the absence of cell division** as assessed by incorporation of BrdU, excluded the possibility of selective survival or overgrowth by a small contaminating population of centroacinar-ductular cells. (. . .) The present results are indicative of a process termed "direct transdifferentiation", in which **cellular phenotypic conversion is independent from DNA-replication**. (Rooman, page 912, 1st paragraph of Discussion, emphasis added.)

6. Therefore, the expansion of exocrine cells into ductal cells, which is the phenomenon disclosed by Gmyr, does not involve cell proliferation. Rather, the expansion of exocrine cells into ductal cells is a mechanism in which exocrine cells acquire characteristics of ductal cells during culture *in vitro* without passing through a proliferative (cell division) phase, as required by the claims. Nothing in Gmyr indicates that the exocrine cells were allowed to proliferate, as recited in the claims. Nor does Gmyr suggest that proliferation is required or desirable for the expansion of ductal cells, much less for the production of islet cells from exocrine or ductal tissue, as recited in the claims.

7. In addition, the Examiner's belief that the expression of cytokeratin 19 indicates proliferation and loss of differentiation equivalent to the dedifferentiated cells of the claims is incorrect. The expression of cytokeratin 19 (as described in Gmyr) is a marker for ductal epithelial cells. Cytokeratin 19 is not a marker for proliferating cells that have lost their ductal phenotype. This much is clear from Gmyr itself, which states: "Immunohistochemical studies confirmed the ductal epithelial (cytokeratin 19, CA19-9) nature of the cells in each of the three methods" (emphasis added). See also Bouwens et al. (1995) Identification of rat pancreatic duct cells by their expression of cytokeratins 7, 19 and 20 in vivo and after isolation and culture. *J. Histochem. Cytochem.* 43:245-53 (abstract enclosed). Therefore, the fact that the Gmyr cells express cytokeratin 19 merely confirms that the cells produced by the Gmyr method are ductal cells.

8. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under §1001 of Title XVIII of the United States

Code, and that such willful false statements may jeopardize the validity of this Application for Patent or any patent issuing thereon.

Susan Bonner-Weir

Susan Bonner-Weir, Ph.D.

January 6, 2004
Date

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